High Prevalence of Primary Antibiotic Resistance in *Helicobacter pylori* Isolates in Italy

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**Abstract**

**Background & Aims**: *H. pylori* eradication with standard therapies is decreasing worldwide, mainly because of increased primary antibiotic resistance rates. We aimed to assess primary resistance in *H. pylori* isolates towards the most used antibiotics in clinical practice. **Methods**: The study enrolled consecutive, dyspeptic, adult patients, never treated for *H. pylori*, who had underwent upper endoscopy. Gastric biopsies were taken for standard histology, and two further antral biopsies were used for *H. pylori* culture. Minimal inhibitory concentrations (MIC) ≥1, ≥8 and ≥1 mg/L were used as break point for clarithromycin, metronidazole, and levofloxacin resistance, respectively. **Results**: Bacterial culture was successful in 145 (94%) out of 154 infected patients. Resistance towards at least one antibiotic was detected in 111 (76.6%) isolated, and multiple antibiotic resistance in 35.2% of cases. Primary resistance towards clarithromycin, metronidazole, and levofloxacin was detected in 51 (35.2%), 86 (59.3%), and 32 (22.1%), respectively. Levofloxacin resistance was significantly associated with the male sex (OR: 11.3, 95% CI = 1.2-103; P = 0.03), whilst females were at a higher risk of being infected with bacterial strains harbouring a double clarithromycin and metronidazole resistance (OR: 4.9, 95% CI = 1.2-19.8, P = 0.02). **Conclusion**: Our data indicate a very high primary resistance rate towards the most used antibiotics in *H. pylori* isolates. The efficacy of standard eradication therapies is expected to further decrease in the next years.

**Key words**


**Introduction**

Although *Helicobacter pylori* prevalence is declining in developed countries [1], millions of people are still infected worldwide. Such an infection may cause different benign gastroduodenal diseases – such as non-ulcer dyspepsia-or peptic ulcer and malignant diseases, including gastric lymphoma and carcinoma [2-4]. Moreover, both idiopathic thrombocytopenic purpura and idiopathic iron deficiency anaemia are significantly associated with *H. pylori* infection [5, 6]. Achieving *H. pylori* eradication remains a challenge for physicians, as there is no therapy regimen able to cure the infection in all treated patients. Although different factors may be involved in treatment failure [7], primary resistance towards antibiotics play a pivotal role. Indeed, resistance to either clarithromycin or metronidazole significantly reduces the success rate of standard therapies [8]. Moreover, some observations suggest that primary levofloxacin resistance in *H. pylori* isolates is also increasing [9]. Therefore, to monitor primary antibiotic resistance towards the most used antibiotics in either first- and second-line therapy is clinically relevant.

The present study was designed to update data on primary antibiotic prevalence in consecutive *H. pylori* isolates from Italian patients, and to search for potential risk factors for antibiotic resistance.

**Methods**

**Patients**

All consecutive patients aged over 18 years who had underwent upper endoscopy in a single centre (Bologna) between January 2010 and December 2011 for dyspeptic symptoms were considered for enrolment. Patients were
excluded if they had been previously treated for *H. pylori* infection, or had taken either proton pump inhibitors or antibiotics in the 4 weeks preceding the study. For the purpose of the study, patients with either a peptic ulcer (ulceration ≥5 mm in diameter) or mucosal erosions (superficial lesion <4 mm) in the stomach or duodenum were grouped together (PUD), whilst non-ulcer dyspepsia was diagnosed when no macroscopic lesions were detected at endoscopy (NUD). All patients underwent endoscopy with biopsies for histology (two samples from the antrum, two from the incisura angularis, and two samples from the corpus), as routinely performed in our Centres. Further biopsies (two samples from the antrum) were taken for bacterial culture. Smoking habit, alcohol use, and body mass index (BMI) were registered for each patient. All participants gave written informed consent to participate.

**Bacterial culture**

Biopsies collected for bacterial culture were immediately streaked onto selective medium Pylori Agar (BioMérieux Italia, s.p.a.); the plates were incubated under microaerobic conditions at 37°C for 72 h. Once incubated, the colonies resembling *H. pylori* were identified by Gram stain and by oxidase, catalase and urease tests. Suspensions from colonies resembling *H. pylori* were prepared in sterile saline solution and by a microaerobic atmosphere at 37°C for 72 h. Isolated strains were streaked in three directions with a swab dipped into each bacterial suspension to produce a lawn of growth, an E-Test strip (E-Test; AB Bio disk, Solna, Sweden) was placed each onto a separate plate, which was immediately incubated in a microaerobic atmosphere at 37°C for 72 h. Isolated strains were tested for primary clarithromycin, metronidazole and levofloxacin resistance using as the breaking point the minimal inhibitory concentration (MIC) ≥1, ≥8 and ≥1 mg/L, respectively.

**Statistical analysis**

For comparison between subgroups, the Chi-squared test, Fisher’s exact test, and t-test were used as appropriate. A multivariate analysis was performed to search for factors potentially involved in bacterial resistance. A P value <0.05 was considered statistically significant.

**Results**

Overall, *H. pylori* bacterial culture was successful in 145 (94%) out of 154 consecutive patients with the infection detected at histology. There were 55 males and 90 females, the median age was 51 years (range: 24-83); 36 (24.8%) were smokers, 22 (15.2%) alcohol users, and their mean body mass index (BMI) was 24.6±3.9 kg/m².

As shown in Table I, resistance towards at least 1 antibiotic was detected in 111 (76.6%), including 60 strains with a single resistance, and 51 with a multiple resistance. In detail, the overall primary resistance towards clarithromycin, metronidazole, and levofloxacin was detected in 51 (35.2%), 86 (59.3%), and in 32 (22.1%), respectively.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible strains</th>
<th>Resistant strains*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>11 (7.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td>41 (28.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>8 (5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin plus metronidazole</td>
<td>27 (18.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin plus levofloxacin</td>
<td>6 (4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin plus metronidazole</td>
<td>7 (4.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole plus levofloxacin</td>
<td>11 (7.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*At least 1 antibiotic resistance; BMI: body mass index; PUD: peptic ulcer disease; NUD: non-ulcer dyspepsia.

At univariate analysis, patients infected with susceptible and resistant strains towards at least 1 antibiotic did not differ for all the considered factors (Table II). Similarly, no difference emerged when each antibiotic was singularly considered (data not shown). The multivariate analysis showed that levofloxacin resistance was significantly associated with the male sex (OR: 11.3, 95% CI = 1.2-103; P = 0.03), whilst females were at a higher risk of being infected with bacterial strains harbouring a double clarithromycin and metronidazole resistance (OR: 4.9, 95% CI = 1.2-19.8; P = 0.02).

**Discussion**

Although *H. pylori* treatment failure may be due to different causes, primary resistance towards different antibiotics remains the main factor [5]. Antibiotic resistance rates are increasing worldwide [8], and the cure rate following standard eradication therapies is distinctly lower when either clarithromycin or metronidazole resistance is present [6]. Levofloxacin is generally used as a second-line or rescue therapy, but its inclusion in first-line therapy has been proposed in some recent trials [10]. Therefore, to update data on primary *H. pylori* resistance towards these widely used antibiotics is relevant for clinical practice.

The present study found a very high resistance rate towards clarithromycin (35.2%), metronidazole (59.3%), and levofloxacin (22.1%) in *H. pylori* isolates. It is worth noting that resistance rates were 16.9%, 29.4%, and 19.1%, respectively.
respectively, in bacterial strains cultured between June 2004 and June 2006 from the same area [8]. Therefore, primary resistance towards both clarithromycin and metronidazole has distinctly increased in the last few years, whilst levofloxacin resistance has remained stably high. Of note, we also observed that multiple resistance rate was as high as 35.2%, clarithromycin-metronidazole simultaneous resistance being the most frequently detected, which was higher than 10.6% we found in the past [8]. Using multivariate analysis, levofloxacin resistance was significantly associated with the male sex, while female patients had a higher probability of being infected with clarithromycin-metronidazole double resistant strains.

All these observations would have an impact when choosing the first-line therapy, which should be based on the prevalence of primary clarithromycin and metronidazole resistance, as suggested in current European guidelines [11].

Conclusion

We found a very high primary resistance rate towards the most frequently used antibiotics in H. pylori isolates. Therefore, the efficacy of standard eradication therapies is expected to further decrease in the future.

Conflicts of interest

None to declare.

References